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Full Length Research Paper

Effect of compost action on the antimicrobial activity of the leaves of *Ocimum gratissimum* (L.) and *Gongronema latifolium* (Benth)

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The effect of fertilizer treatment on the antimicrobial activity of the leaves of *Ocimum gratissimum* (L.) and *Gongronema latifolium* (Benth) was investigated. Cultivated *O. gratissimum* (L) and *G. latifolium* were applied with NPK (15:15:15) fertilizer at 100, 200, 300, 400 and 500 kg/ha treatment levels in planting buckets derived using the furrow slice method two months after seedling emergence. No fertilizer treatment served as control. Leaves were harvested one month after treatment. The ethanolic extracts of the harvested leaves were used to determine the sensitivity of the extracts on *Klebsiella pneumonia, Escherichia coli, Staphylococcus aurues, Pseudomonas aeruginosa, Salmonella typhi, Streptococcus faecalis, Candida albicans* and *Aspergillus niger.* The result obtained showed that the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium* was significantly ($P < 0.05$) increased by fertilizer treatment. The inhibition zone increased with increase in the level of fertilizer treatment. The ethanolic extracts of both plants whether treated or not had no antimicrobial effect on *A. niger*. This research revealed that fertilizer treatment might have increased the phytochemical content of the leaves of the plants which in turn enhanced their antimicrobial potential.

Key words: Pathogens, NPK fertilizer, ethanolic extracts, inhibition zone.

INTRODUCTION

The role of plants in the maintenance of good health has been reported (Burkill, 1995; Moerman, 1996). In Nigeria, these indigenous plants contain bioactive compounds that exhibit physiological activities against bacteria and other microorganisms and are also used as precursor for the synthesis of useful drugs (Edeoga et al., 2003; Okwu, 2001; Osuagwu et al., 2007; Osuagwu, 2008; Sofowara, 1993). The antimicrobial activities of these plants and their products such as essential oils are well documented (El-Zaher et al., 2006; Ijeh et al., 2006; Mevy et al., 2007; Sahraoui et al., 2007; Vagionas et al., 2007). Thus, these plants are therefore used in the treatment of many diseases such as rheumatism, diarrhea, malaria, elephantiasis, cold obesity, dysentery, high blood pressure, malnutrition, gonorrhea and others (Batram, 1998; Burkill, 1995; Gill, 1992).

The biosynthesis of these bioactive plant chemicals is influenced by various agronomic and environmental factors. Fertilizer treatment is known to determine the concen-tration of these compounds in plants (Asami et al., 2003; Khalil et al., 2007; Osuagwu and Nwachukwu, 2007; Rasmussen et al., 2008; Saradhi et al., 2007). Water stress (drought) is also reported to influence the concen-tration of these phytochemicals in plants (Selmar, 2008; Zheng et al., 2006).

The antimicrobial activities of *Ocimum gratissimum* and *Gongronema latifolium* have been investigated and reported. The antimicrobial activities of *O. gratissimum* have been documented (Ezekwesili et al., 2004; Ijeh et al., 2005; Iwalokun et al., 2003; Matasyoh et al., 2007; Mbata and Salkia, 2007; Tchoumbougnong et al., 2005) and that of *G. latifolium* reported (Afolabi and Eleyinmi, 2007). The objective of this research is to ascertain the implication of fertilizer treatment on the antimicrobial activity of the

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Table 1. Physical properties of the soil.

Properties	Values
Particle size distribution	
Sand	70.90%
Silt	15.40%
Clay	13.70%
Texture	5 L
pH (H_2O)	5.01
Organic carbon (%)	0.75%
Organic matter (%)	1.29%
Available phosphorus (mg/Kg)	46.00
Total nitrogen (%)	0.08%
Exchangeable bases (mg/100g)	
Ca^{++}	2.40
Mg^{++}	2.00
K^{+}	0.07
Na^{+}	0.23
Exchangeable acidity (ME/100g)	1.20
Effective cation exchange capacity (ME/100g)	5.90

leaves of *O. gratissimum* and *G. latifolium*.

MATERIALS AND METHODS

Plant sample

The seeds of *O. gratissimum* were collected from a homestead garden in Amaogwu village Bende town, Bende Local Government Area of Abia State. The fresh and succulent stem cuttings of *G. latifolium* were obtained from the forest strip of the Forest Department, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. Both plant materials were identified by the taxonomic unit of the Botany section of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The seeds of *O. gratissimum* were raised into seedling in nursery boxes before they were transplanted into planting buckets. Stem cuttings of *G. latifolium* were planted directly into the planting buckets.

Cultivation of the plants was carried out using 24 plastic buckets containing 8 Kg of sterilized soil. The soil used for the research was analyzed to determine the physiochemical properties (Table 1).

Treatments were carried out in four replicates of each treatment. The inorganic fertilizer (NPK 15:15:15) used for the study was obtained from the store of the Abia State Ministry of Agriculture, Umuahia, Abia State. Five levels of fertilizer treatments 100, 200, 300, 400 and 500 Kg/ha derived using the furrow slice method (Brady and Weil, 1999), in four replicates was used. No fertilizer treatment served as control. Treatment occurred two months after seedling emergency. Harvesting of plants leaves for antimicrobial activity investigation was carried out one month after treatment.

Determination of antimicrobial activity

Preparation of plant extract

The ethanolic extracts of the leaves of *O. gratissimum* and *G. latifolium* was prepared using the method of Ijeh et al. (2005). Fifty grams of the pondered samples was soaked in 200 ml of absolute ethanol and allowed to stand for 24 h. It was filtered using a Whatman (No. 1) filter paper. The filtrate was evaporated to dryness over steam bath. The residue was dissolved in deionized water to obtain the desired plant extract for the antimicrobial tests.

Preparation of innocula

Klebsiella pneumonia, Escherichia coli, Staphylococcus aurues, Pseudomonas aeruginosa, Salmonella typhi, Streptococcus faecalis, Candida albicans and *Aspergillus niger* used in the research were obtained from the stock culture of the Microbiology Laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria. Viability test of each isolate was carried out by resuscitating the organisms in buffered peptone broth and thereafter, sub-cultured into nutrient agar medium and incubated at 37°C for 24 h.

Antimicrobial test

The sensitivity of the test organisms to the ethanolic extracts of the leaves of *O. gratissimum* and *G. latifolium* was carried out using the diffusion method described by Ebi and Ofoefule (1997).

20 ml of the molten nutrient agar was seeded with 0.2 ml of broth culture of the test organisms in sterile Petri dishes. The Petri-dishes were rotated slowly to ensure a uniform distribution of the organisms. They were left to solidify and in the dish cups of 8.0 mm diameter were made in the agar using a sterile Pasteur pipette. The Petri-dishes were allowed to stand for about 30 min at room temperature to allow for proper diffusion of the extracts to take place. The plates were then incubated at 37°C for 24 h. The zone of inhibition in millimeter were measured and recorded. The test was carried out in the laboratory of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

Statistical analysis

The design for the research was complete randomized design in four replicates of each treatment. Analysis of variance (ANOVA) was used to analyze the data and LSD at 0.05 probability level was used to determine the difference among treatments.

RESULTS AND DISCUSSION

Fertilizer treatment significantly ($P < 0.05$) affected the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium* (Tables 2 – 5).

There was significant increase ($P < 0.05$) in the ability of the leaves of *O. gratissimum* to inhibit the activity of *K. pneumonia, E. coli, S. aurues, P. aeruginosa, S. typhi, S. faecalis* and *C. albicans* due to fertilizer treatment (Tables 2 and 3). Fertilizer treatment also significantly ($P < 0.05$) increased the ability of the leaves of *G. latifolium* to inhibit the activity of *K. pneumonia, E. coli, S. aurues, P. aeruginosa, S. typhi, S. faecalis* and *C. albicans* (Tables 4 and 5).

The observed increased ability of the leaves of *O. gratissimum* and *G. latifolium* to inhibit the microbial activity of these pathogens as testified by the inhibition zones in response to fertilizer treatment might be related to the enhanced synthesis and accumulation of phyto-

Table 2. Effect of NPK fertilizer treatment of the antimicrobial activity of the leaves of *O. gratissimum* on *K. pneumonia, E. coli, S. aurues* and *P. aeruginosa.*

Treatment	Zone of inhibition (mm)			
	K. pneumonia	***E. coli***	***S. aurues***	***P. aeruginosa***
Control	20.00^{a}	26.00^{a}	13.00^{a}	16.00^{a}
100 kg/ha	25.00^{b}	28.00^{ab}	21.00^{b}	16.00^{ab}
200 kg/ha	27.00^{bc}	30.00^{bc}	24.00^{bc}	20.00^{bc}
300 kg/ha	29.00^{c}	32.00^{cd}	26.00^{cd}	23.00^{cd}
400 kg/ha	32.00^{d}	34.00^{de}	28.00^{cd}	24.00^{d}
500 kg/ha.	34.00^{d}	36.00^{e}	29.00^{d}	26.00^{a}
LSD (< 0.05)	1.414	1.810	1.863	1.691

Mean of four replicates. Different letters in the superscript in the same row indicates significant difference (P < 0.05).

Table 3. Effect of NPK fertilizer treatment on the antimicrobial activity of the leaves of *O. gratissimum* on *S. typhi, S. faecalis, C. albicans* and *A. niger*.

Treatment	Zone of inhibition (mm)			
	S. typhi	***S. faecalis***	***C. albicans***	***A. niger***
Control	17.00^{a}	15.00^{a}	7.00^{a}	0.00
100 kg/ha	20.00^{ab}	18.00^{b}	12.00^{bc}	0.00
200 kg/ha	22.00^{bc}	21.00^{b}	14.00^{c}	0.00
300 kg/ha	25.00^{cd}	22.00^{bc}	10.00^{ab}	0.00
400 kg/ha	27.00^{d}	25.00^{cd}	12.00^{bc}	0.00
500 kg/ha.	28.00^{d}	27.00^{d}	13.00^{bc}	0.00
LSD (< 0.05)	1.599	1.740	1.599	

Mean of four replicates. Different letters in the superscript in the same row indicates significant difference (P < 0.05).

Table 4. Effect of NPK fertilizer treatment on the antimicrobial activity of the leaves of *G. latifolium* on *K. pneumonia, E. coli, S. aurues* and *P. aeruginosa.*

Treatment	Zone of inhibition (mm)			
	K. pneumonia	***E. coli***	***S. aurues***	***P. aeruginosa***
Control	13.00^{a}	15.00^{a}	15.00^{a}	10.00^{a}
100 kg/ha	20.00^{b}	25.00^{b}	23.00^{b}	17.00^{b}
200 kg/ha	24.00^{c}	27.00^{cd}	26.00^{bc}	21.00^{c}
300 kg/ha	26.00^{cd}	30.00^{cd}	25.00^{bc}	20.00^{bc}
400 kg/ha	28.00^{d}	32.00^{cd}	28.00^{cd}	25.00^{d}
500 kg/ha.	32.00^{e}	35.00^{e}	30.00^{d}	28.00^{d}
LSD (< 0.05)	1.546	1.740	1.732	1.683

Mean of four replicates. Different letters in the superscript in the same row indicates significant difference (P < 0.05).

chemicals (alkaloids, phenols, saponins, steroids, tannins and other plant chemicals substances) by these plants a consequence of fertilizer treatment. This in turn increased the ability of the extracts of the leaves to inhibit the activity of these pathogens. Enhanced production of phytochemicals, vitamins and other plant chemical substances by plants as a result of fertilizer treatment has been reported (Das et al., 2006; Koloziej, 2007; Mozaffar, 1994; Osuagwu and Nwachukwu, 2007; Osuagwu, 2008; Omoidbaigi et al., 2008; Polat et al., 2008; Silva et al., 2006; Zheng et al., 2006).

The leaf extracts of *O. gratissimum* and *G. latifolium* had no antimicrobial effect on *A. niger* . The leaves of both treated and untreated plants did not inhibit the microbial activity of *A. niger*. This indicates that *A. niger* is resistant to the chemicals contained in the leaves of the plants. The extracts of the leaves of *O. gratissimum* and *G. latifolium* had more inhibitory effect on the microbial

Table 5. Effect of NPK fertilizer treatment on the antimicrobial activity of the leaves of *G. latifolium* on *S. typhi, S. faecalis, C. albicans* and *A. niger.*

Treatment	Zone of inhibition (mm)			
	S. typhi	***S. faecalis***	***C. albicans***	***A. niger***
Control	12.00^{a}	14.00^{a}	5.00^{a}	0.00
100 kg/ha	24.00^{b}	22.00^{b}	14.00^{b}	0.00
200 kg/ha	26.00^{bc}	25.00^{c}	16.00^{bc}	0.00
300 kg/ha	22.00^{b}	22.00^{b}	16.00^{b}	0.00
400 kg/ha	26.00^{bc}	24.00^{b}	16.00^{bc}	0.00
500 kg/ha.	29.00^{c}	26.00^{bc}	19.00^{c}	0.00
LSD (< 0.05)	1.764	1.856	1.683	

Mean of four replicates. Different letters in the superscript in the same row indicates significant difference (P < 0.05).

activity of bacteria, when compared with the effect on fungi (Tables 2 – 5). The above observation relates to the reactions of different organisms to similar and different environmental conditions.

Furthermore, generally, increasing the level of fertilizer treatment led to corresponding increase in the ability of the leaves of the plants to inhibit the microbial activity of the pathogens, which in turn affect the functions of the quantity of phytochemicals they contain. Wiesler (1997) observed that the impact of nitrogen fertilizer on metabolism of plants depends on factors such as its concentration in the soil and chemical form.

The importance of the finding from this research is that the application of NPK fertilizer at the appropriate levels will enhance the antimicrobial activity of the leaves of *O. gratissimum* and *G. latifolium* and thus, increase their value and efficacy as medicinal plants.

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